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Research and Development

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Project Summary

ORIGIN

Evaluation of the Tentative Standard Method for Enteric Virus Concentration and Detection

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The tentative *Standard Method* (TSM) for concentrating enteric viruses from large volumes of drinking water and other finished waters was evaluated for nine enteric viruses using 100-gal (378-l) tap water volumes experimentally contaminated with about 100 plaque-forming units (PFU) of virus. The mean recoveries of poliovirus 1, coxsackieviruses A9 and B3, echovirus 7, bovine enterovirus 1, Minute Virus of Mice (parvovirus), reovirus 3, adenovirus 5 and simian adenovirus 11 were 22, 25, 2.5, 6.7, 22, 5.0, <1, <1 and <1%, respectively when the viruses were evaluated individually in succession. Although the recovery differences for the five enteroviruses suggested variable TSM effectiveness depending upon virus type, subsequent TSM experiments in which these viruses were simultaneously tested showed no significant recovery differences, averaging 16% (±2.7, 95% CI) overall. TSM recoveries for purified, dispersed mixtures of enteroviruses obtained from raw sewage and seeded into 100-gal volumes of tap water averaged only 3.9% which was approximately the same as poliovirus recoveries during the same time period.

Enterovirus recoveries showed marked seasonal variations that may be related to water quality changes. Reduced virus recovery was partly due to interference with virus adsorption to filters caused by soluble or colloidal tap water constituents that were removable by granular activated carbon. The effects of these interferences could be overcome by adding aluminum or magnesium chloride to the tap water to enhance virus adsorption.

Low virus recoveries by the TSM were also due to virus inactivation by the pH 11.5 glycine-NaOH used for eluting adsorbed viruses from filters. In this eluent the T-90 values (time for 90% virus inactivation) for Minute Virus of Mice, poliovirus, reovirus and the simian adenovirus SV-11 were 20, 1.8, <1 and <1 minutes, respectively.

Virus recoveries with TSM and other negatively charged filters could be markedly improved by various procedural modifications: (1) the use of aluminum or magnesium chloride to enhance virus adsorption, (2) the use of less alkaline glycine or beef extract both with and without EDTA for elution of viruses from primary adsorbents, and (3) recirculation of the eluent through the adsorbent filters. With the exception of MVM, viruses in beef extract eluents could be efficiently reconcentrated by organic flocculation.

Improved virus recoveries were also obtained with adsorbent filters that are more electropositive than TSM filters. Zeta Plus filters that are

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positively charged up to about pH 5 to 6 adsorbed ≥99% of various viruses from tap water at ambient pH levels of 7.0-7.5 without added polyvalent cation salts. Most viruses could be effectively eluted with glycine-NaOH, pH ≥9.5 or beef extract. Low levels of poliovirus in 100-gal (378-l) volumes of tap water were more effectively recovered with Zeta Plus filters than with the TSM using Cox filters.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction and Background

The potential transmission of human enteric viruses via drinking water and other waters is of considerable public health concern. The viruses of major concern in water are those that infect the alimentary tract of man and in some cases animals, and are excreted in large quantities with the feces by infected persons. These are the so-called enteric viruses, and they include members of the following taxonomic groups: (1) enteroviruses, (polio-, coxsackie- A and B and echoviruses), (2) reo- and rotaviruses, and (3) adenoviruses as well as the Norwalk-type agents and Hepatitis type A, which are not yet taxonomized due to limited information on morphology and chemical composition.

The continued occurrence of waterborne outbreaks of Hepatitis A and perhaps other virus diseases such as gastroenteritis as well as the apparent isolation of enteric viruses from drinking water supplies in the U.S. and elsewhere, indicate the need for a more thorough and systematic evaluation of the potential for virus disease transmission via drinking water. Evaluation of this problem requires the development and utilization of simple and reliable methods to quantitatively detect relatively small amounts of enteric viruses in large volumes of raw and finished drinking water supplies.

Research efforts on methods to detect enteric viruses in water have produced two-step microporous filter adsorption-elution methods that are sometimes capable of concentrating small numbers of enteroviruses from 100 or more gallons of drinking water, and a tentative method of this type was introduced into the 14th edition of *Standard Methods*.

In this method the water is pressure filtered through microporous (0.45μ to 8.0 μm pore size) filters having large surface areas to which viruses adsorb by a reversible reaction. The adsorption reaction is thought to be primarily electrostatic in nature, although weak attractive forces, such as hydrogen bonding, dipole-dipole interactions and hydrophobic effects may also be involved. In order to enhance virus adsorption to the filter sufaces, appropriate ionic conditions are created in the flowing water by the controlled addition of acid and sometimes polyvalent cation salts prior to passage of the water through the adsorbent filter. After processing the desired volume of water, the adsorbed viruses that have accumulated on the filter surfaces are eluted with approximately one liter of an alkaline buffer solution consisting of pH 11.5 glycine-NaOH. The eluate obtained is readjusted by the addition of acid and $AlCl_3$ to ionic conditions that enhance virus adsorption: pH 3.5 and .0005 M $AlCl_3$. The viruses are then adsorbed to a second, smaller (47-mm diameter) microporous filter series composed of fiberglass, asbestos and epoxy. The adsorbed viruses are then eluted from this filter with several milliliters of alkaline buffer solution. The virus-containing eluate is adjusted to a physiological pH and isotonicity and then assayed for viruses in cell cultures using conventional virological techniques.

The development of flow-through filter adsorption-elution systems for concentrating small quantities of viruses from large volumes of water represents a much needed technical achievement. However, many of the key variables involved in this type of methodology have not been adequately evaluated, and some of the procedures and equipment in current use have been somewhat arbitrarily selected. A rational and systematic evaluation of the methodology and materials is needed.

In particular, it has not been demonstrated that the tentative Standard Method (TSM) is capable of efficiently detecting viruses of all major enteric virus groups including Hepatitis A virus. The method was developed and evaluated primarily for enteroviruses, especially poliovirus, and there is information in the literature indicating that the method may be incapable of recovering some other enteric viruses. For example, in the application of filter adsorption-elution methods to adenoviruses, one study found that adenovirus type 5 was inactivated by the alkaline glycine-NaOH used for elution of adsorbed viruses.

Another deficiency concerning evaluation of the TSM is that the method has not been evaluated over a sufficient time period with the same viruses and water source to determine if changes in water quality influence its effectiveness. Dissolved and colloidal substances in water may interfere with virus recovery, and the types and concentrations of such interfering substances may vary over time.

Objectives

General Objective

To rigorously and systematicall evaluate the tentative Standard Methc for concentrating and detecting enter viruses in large volumes of drinkir water and other finished waters.

Specific Objectives

To evaluate the tentative *Standar Method* for detecting and concentratin enteric viruses from tap water using th four different types of microporous filte systems that are currently recommende as virus adsorbents and representativ viruses from the major enteric viru groups. The four primary virus adsorben systems being evaluated are: (a) Balstor fiberglass-epoxy filter tubes, (b) Co fiberglass-asbestos-epoxy filter discs (c) Millipore cellulose nitrate mem branes, and (d) Commercial Filters fiberglass cartridge filters and Cox fiberglass-asbestos-epoxy filter discs in series. A total of nine different viruses are being tested: poliovirus, coxsackie-viruses A9 and B3, echovirus 7, bovine enterovirus 1, reovirus 3, simian adenovirus SV-11, (human) adenovirus 5 and Minute Virus of Mice (an enteric parvovirus).

After evaluating the tentative Standard Method with the nine different laboratory strains of enteric viruses, the method will then be evaluated using mixtures of naturally occurring enteric viruses obtained from sewage.

If the present tentative Standard Method is found to be incapable of efficiently recovering certain enteric viruses, then modifications of the presently recommended methodology will be evaluated for their ability to efficiently detect representatives of all enteric virus groups.

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Conclusions

1. The current tentative Standard Method (TSM) for enteric virus concentration from drinking water and other finished waters has serious deficiencies causing it to give poor virus recoveries.
2. One major deficiency of the TSM is that the pH 11.5 glycine-NaOH used to elute adsorbed viruses from filters rapidly inactivates adenoviruses and reoviruses. It also inactivates enteroviruses and parvoviruses, but not as rapidly. Alternative eluents which employ less alkaline (pH 9.5-10.5) conditions in glycine, proteinaceous media such as 0.3-3% beef extract, chelating agents such as EDTA, or combinations of these conditions show considerable promise for all major enteric virus groups. Virus elution efficiency is further improved when these alternative eluents are recirculated through the adsorbents, thereby increasing the contact time.
3. Another serious deficiency of the TSM causing reduced virus recoveries is that soluble and/or colloidal substances in finished tap water can interfere with virus adsorption to conventional, electronegative TSM primary virus adsorbents. There appear to be temporal changes in magnitude of these interfering effects that may be related to seasonal changes in the nature and amount of interfering substances in tap water. The effects of these interfering substances can be overcome by adding polyvalent cation salts, such as $MgCl_2$ and $AlCl_3$, to the tap water to enhance virus adsorption to TSM primary virus adsorbents and the Filterite pleated cartridge fiberglass-epoxy filter.
4. The four alternative primary virus adsorbents of the TSM do not differ significantly in terms of virus recovery efficiency, although recoveries with the Balston filter were frequently lower than those with the other three adsorbent systems. The primary virus adsorbent systems did differ in terms of their susceptibilty to clogging, with the Millipore and Carborundum-Cox systems being more susceptible than the Balston and Cox systems.
5. The results of this study suggest that all enteroviruses are apparently recoverable with equal effectiveness by the TSM. This conclusion is based on finding: (1) that the recovery efficiencies for five different laboratory-grown enteroviruses did not differ significantly in simultaneous experiments and (2) that recovery efficiencies for natural enterovirus mixtures obtained from sewage were not significantly different from recovery efficiencies for exogenously added, laboratory-grown poliovirus during the same time period.
6. Microporous filters that are more electropositive than the current TSM filters and are composed of diatomaceous earth-cellulose-"charge modified" resin mixtures appear to offer distinct advantages as virus adsorbents because: (1) they efficiently adsorb viruses from tap water at neutral pH without added polyvalent cation salts, (2) they are less affected by soluble and/or colloidal interferences in tap water that reduce virus adsorption, and (3) with the possible exception of adenoviruses, adsorbed viruses can be readily eluted with either pH 9.5 to 10.5 glycine-NaOH or 0.1 to 3% beef extract, pH 9 to 10.
7. Highly electropositive microporous filters composed of asbestos-cellulose mixtures may also be effective virus adsorbents because they efficiently adsorb viruses over a wide pH range without added salts and the adsorbed viruses can be eluted with pH 9 to 10 beef extract.

Recommendations

1. Because the current TSM for concentrating enteric viruses from drinking water and other finished waters is relatively ineffective, improved filter adsorption-elution methods that will be suitable and effective in field use should be developed and evaluated.
2. Further efforts should be made to develop and evaluate alternative eluents to pH 11.5 glycine-NaOH. In particular, the use of glycine-NaOH at lower pH levels and 0.1 to 3% beef extract with and without chelating agents such as EDTA or other additives should be investigated.
3. Further studies should be done with beef extract and other proteinaceous eluents to improve the organic flocculation procedure for parvoviruses.
4. Because polyvalent cation salts such as $MgCl_2$ and $AlCl_3$ are capable of overcoming the effects of the soluble and/or colloidal interferences in tap water that reduce virus adsorption efficiency, it may be prudent to routinely use salt enhancement of virus adsorption, unless it can be experimentally demonstrated the efficient virus recoveries from large volumes of a particular water are obtained without added salts.
5. Electronegative fiberglass-epoxy pleated cartridge filters (Filterite) should be considered as a fifth alternative primary virus adsorbent because: (1) they have a large surface area and, hence, a large filtration-volume capacity for water; (2) they efficiently adsorb viruses under the same conditions as TSM filters, and (3) the adsorbed viruses are elutable with pH 10.5 glycine-NaOH and beef extract.
6. Additional studies should be done to further develop and evaluate improved and simplified filter adsorption-elution methods for virus concentration from water based on the use of electropositive adsorbent filters.
7. The physico-chemical mechanisms of virus adsorption to and elution from filters and other surfaces should be investigated in order to provide a rational basis for the further development and optimization of filter adsorption-elution methods to concentrate enteric viruses from water. In addition, the chemical characteristics and behavior of the dissolved and/or colloidal substances in tap water that interfere with virus adsorption to filters should also be fully investigated in order to develop optimum methods for their elimination or control.

The results of this investigation have appeared in three publications: "Concentration of Poliovirus from Tap Water Using Positively Charged Microporous Filters" by M.D. Sobsey and B.L. Jones, Appl. and Environ. Microbiol. 37(3):588-95 (Mar. 1979); "Evaluation of the Tentative Standard Method for Enteric Virus Concentration from Large Volumes of Tap Water" by M.D. Sobsey et al., J. AWWA 72(5): 292-99 (May 1980); and "Modifications of the Tentative Standard Method for Improved Virus Recovery Efficiency" by M.D. Sobsey et al., J. AWWA 72(6):350-55 (June 1980).

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The complete report, entitled "Evaluation of the Tentative Standard Method for Enteric Virus Concentration and Detection," (Order No. PB 82-108 416; Cost: $9.50, subject to change) will be available only from:
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